Measurement of ageing effects on muscle tone and mechanical properties of rectus femoris and biceps brachii in healthy males and females using a novel hand-held myometric device

Agyapong-Badu, Sandra; Warner, Martin; Samuel, Dinesh; Stokes, Maria

DOI:
10.1016/j.archger.2015.09.011

Note: this is the final draft of the article:

Agyapong-Badu S. Warner M, Samuel D, Stokes M.
Measurement of ageing effects on muscle tone and mechanical properties of rectus femoris and biceps brachii in healthy males and females using a novel hand-held myometric device. *Arch Gerontol Geriatr* 2015 In Press
doi:10.1016/j.archger.2015.09.011

Accepted 28th September 2015 for publication in *Archives of Gerontology and Geriatrics*

The final, fully proofed and peer-reviewed journal article will be available from the publisher online, via the following link:

http://www.journals.elsevier.com/archives-of-gerontology-and-geriatrics/

Accepted 28th September 2015

Title:

Measurement of ageing effects on muscle tone and mechanical properties of rectus femoris and biceps brachii in healthy males and females using a novel hand-held myometric device

Short title:

Ageing effects on muscle tone and mechanical properties

Short title:

Ageing effects on muscle tone and mechanical properties

Authors:

Sandra Agyapong-Badu PhD[a]

Martin Warner PhD[a,b]

Dinesh Samuel PhD[a]

Maria Stokes PhD[a,b]

Affiliations:

[a]Faculty of Health Sciences, Building 45, Highfield Campus, University of Southampton, Southampton, SO17 1BJ, UK

[b]Arthrtis Research UK Centre for Sport, Exercise and Osteoarthritis

Corresponding author:

Prof Maria Stokes PhD FCSP

Faculty of Health Sciences

Building 45, Highfield Campus

University of Southampton

Southampton, SO17 1BJ

United Kingdom

Email: m.stokes@soton.ac.uk

Body of manuscript: 4 tables, 4 figures

Supplementary material: 5 tables as Appendices A-E

Abstract

Background: Age and gender effects on muscle tone and mechanical properties have not been studied using hand-held myometric technology. Monitoring changes in muscle properties with ageing in community settings may provide a valuable assessment tool for detecting those at risk of premature decline and sarcopenia.

Objective: This study aimed to provide objective data on the effects of ageing and gender on muscle tone and mechanical properties of quadriceps (rectus femoris) and biceps brachii muscles.

Methods: In a comparative study of 123 healthy males and females (aged 18-90 years; n=61 aged18-35; n=62 aged 65-90) muscle tone, elasticity and stiffness were measured using the MyotonPRO device.

Results: Stiffness was greater and elasticity lower in older adults for BB and RF ($p<0.001$). Tone was significantly greater in older adults for BB but not for RF when data for males and females were combined ($p=0.28$). There were no gender differences for BB in either age group. In RF, males had greater stiffness (young males 292 vs females 233 N/m; older males 328 vs females 311 N/m) and tone (young 16.4 vs 13.6 Hz; older 16.7 vs 14.9 Hz). Elasticity in RF was lower in young males than females but did not differ between the older groups (both males and females log decrement 1.6).

Conclusions: Stiffness and tone increased with ageing and elasticity decreased. These findings have implications for detecting frailty using a novel biomarker. Age and gender differences are important to consider when assessing effects of pathological conditions on muscle properties in older people.

Key Words: Muscle tone; mechanical properties; Ageing; Muscle stiffness; Muscle elasticity

1. Introduction

Muscle strength is the most commonly used biomarker of musculoskeletal ageing and frailty (Sayer et al. 2006; Cooper et al. 2011b). However, strength measurements are not always feasible in older people due to e.g. pain from arthritis or impaired cognitive function from dementia. Tools for assessing muscle health are needed, that are not influenced by pain or cognition.

Sarcopenia, the age-associated loss of skeletal muscle mass and strength (Cruz-Jentoft et al. 2010; Rosenberg 2011), is present when muscle mass decreases to a level less than expected for a specified age, gender and race (Baumgartner & Waters 2006). Women have lower muscle mass and strength than age-matched males (Narici & Maffulli 2010) but greater fatigue resistance (Wüst et al. 2008). These differences in functional capacity with age and gender reflect differences in muscle fibre-type composition. There is preferential loss of type 2 (fast twitch) fibres with ageing, which are predominantly distributed around the periphery of muscle fascicles (Manta et al. 1995). Type 1 fibres do not change significantly with ageing (Kararizou et al. 2009). Type 2 fibres have greater intrinsic strength and type 1 fibres are characteristic of endurance and slower contraction rate (Wüst et al. 2008).

Tone and mechanical properties of muscles (Blanpied & Smidt 1993) and tendons (Scaglioni et al. 2003) change with ageing and are also altered in neurological conditions, such as stroke and Parkinson's disease (PD). Muscle tensile stiffness increases with ageing, as demonstrated using the resistance to a single stretch (Blanpied & Smidt 1993) and sheer wave elastography (Eby et al. 2015). Stiffness is also reported to increase with hypertrophy due to strength training (Kubo et al. 2010) and with increasing muscle activation (Blanpied & Smidt 1993). Gender differences in muscle stiffness measured using different laboratory based technologies are conflicting in the literature. For example, greater stiffness in males than females was found using the free oscillation technique (Wang et al. 2015), which might be expected with greater strength in males. However, sheer wave elastography demonstrated greater stiffness in females (Eby et al. 2015). Differences may be due to methods used and muscles studied.

It is important to be able to distinguish the underlying effects of ageing from the specific effects of pathology when tone and mechanical properties are assessed in clinical conditions, such as musculoskeletal and neurological disorders. Clinical assessment of tone is subjective, using manual assessment by rating resistance to passive movement on scales such as the Modified Ashworth Scale (MAS) for stroke (Fleuren et al. 2010) and the Unified Parkinson's disease Rating Scale (UPDRS) for PD (Goetz et al. 2003). Laboratory techniques for assessing tone and mechanical properties objectively, such as ultrasound imaging with dynamometry (Narici et al. 1996; Muraoka et al. 2005) and magnetic resonance elastography (Dresner et al. 2001) are not clinically feasible. There is the need for objective, reliable, valid, robust, easy to use and cost effective ways of assessing skeletal muscle tone and mechanical properties in a clinical setting. Components of muscle tone at rest can be classified as neural or non-neural (intrinsic); neural aspects comprise active muscle tension and stretch reflex contractions, while non-neural components comprise passive stiffness and the inherent viscoelastic properties of the tissues (Britton 2004).

Myoton technology (Myoton AS, Estonia) offers an in-vivo, non-invasive measurement of state of tension (non-neural tone) and mechanical properties of individual skeletal muscles, with the added advantage of portability and relatively low cost (Gavronski et al. 2007). It is necessary to establish normative data using the Myoton device to provide reference values for future assessment of skeletal muscle with ageing and in clinical conditions, as studies have indicated the potential use of Myoton technology to aid diagnosis and management of increased mechanical tone and stiffness in stroke (Chuang et al. 2012) and PD (Rätsep & Asser 2011; Marusiak et al. 2012).

The aim of the present study was to establish normative data for resting stiffness, tone and elasticity, and identify age and gender differences in rectus femoris (RF) and biceps brachii (BB) muscles using the MyotonPRO device. It was hypothesised that muscle stiffness and tone would increase, and elasticity decrease, with ageing. Stiffness and tone would also be greater, and elasticity lower, in males than females.

2. Materials and Methods

2.1. Participants

A convenience sample of 123 self-reported healthy participants were studied in four groups: young and older males and females (61 young; aged 18-35 years, recruited from the University, and 62 older participants; aged 65-90 years, recruited from the local community (see Table 1).

Young participants were included if they did not participate in sports or exercise more than three times per week, or competitively at university level or above. For older participants, activity was assessed using the Physical Activity Scale for the elderly (Washburn et al. 1993) to ensure only sedentary or moderately active participants were included.

Exclusion criteria for both age groups were: conditions known to affect muscle characteristics and functional ability; upper and lower limb pathology (fracture, surgery, neoplasm), skin disorder, neurological conditions and musculoskeletal injuries severe enough to require treatment or prevent activity for more than one week in the previous five years. Those taking medications, such as skeletal muscle relaxants, neuromuscular blocking drugs and those unable to understand study requirements were excluded. The study was approved by the Faculty of Health Sciences, University of Southampton Ethics Committee and was conducted in accordance with the Helsinki Declaration of 1975. All participants gave their written informed consent.

Table 1. Participant Characteristics (total n= 123)

	Young males (n=34)	Young females (n=27)	Older males (n=28)	Older females (n=34)
Age (years)	24.5±4.7	26.8±4.7	74.2±5.9	76.1±6.4
Height (m)	1.8±0.1	1.6±0.1	1.7±0.1	1.6±0.1
Weight (kg)	72.6±10	59.6±7.8	78.4±10.4	65.5±8.7
Body mass index (kg/m^2)	23.2±2.5	22.9±2.7	25.9±2.9	25.6±2.7

2.2. Experimental set-up

Participants were tested in supine lying for both muscles and lay relaxed for 10 minutes prior to testing (Bailey et al. 2013). For BB, the participant lay supine with the shoulder externally rotated and elbows extended and wrist supinated. A rolled towel placed under the wrist to flex the elbow approximately 15^0 from the horizontal to take the stretch off the muscle and enable relaxation (Aarrestad et al. 2004). Measurements were taken at a point midway between the most anterior aspect of the lateral tip of the acromion and the mid cubbital fossa, following a gentle, resisted isometric muscle contraction to identify the middle of the muscle belly (Figure 1). Rectus femoris measurement was achieved with the knee extended and hip in neutral, with sandbags placed either side of the ankle to maintain this position (Figure 2). Measurements were taken at two thirds of the distance between the anterior superior iliac spine (ASIS) and the superior pole of the patella, to locate a reproducible site over the muscle belly (Mullix et al. 2012).

Figure 1. MyotonPRO technique for measurement of biceps brachii (BB) stiffness, tone and elasticity

Figure 2. MyotonPRO technique for measurement of rectus femoris (RF) stiffness, tone and elasticity

2.3. Measurement of muscle tone and mechanical properties

All testing was performed by one operator (SAB), an experienced physiotherapist trained in the use of Myoton technology. Training involved a one day introductory course and several practise sessions. Inter-rater reliability was established in a separate study (Agyapong-Badu et al. 2013) and intra-rater reliability on different days was examined in the present study (see below). Measurements were performed on the dominant upper and lower limbs by placing the probe of the device (3mm diameter) perpendicular on the skin over the muscle of interest, with constant preload (0.18N) independently of the investigator, to pre-compress subcutaneous tissues. The device delivered a brief (15ms), low force (0.4N) mechanical impulse, inducing damped natural oscillations of the underlying tissues. These oscillations were recorded by an accelerometer attached to the frictionless measurement mechanism within the device (http://www.myoton.com/en/Technology/Technical-specification). The device then calculated simultaneously the parameters describing resting tone (oscillation frequency [Hz]), elasticity (logarithmic decrement [arbitrary unit]) of damped oscillations and stiffness [N/m] (Gavronski et al. 2007). Two pre-defined measurement series for 10 mechanical impulses at one second apart) were conducted, with a one minute rest between the series.

2.4. Parameters measured

Resting tone or state of tension (frequency [Hz]) defined as the maximum frequency ($F=f_{max}$) computed from the signal spectrum by Fast Fourier Transform (FFT) was recorded. The higher the frequency of dampened oscillations (natural oscillation frequency), the greater the muscle tension, which is increased by contraction (Gavronski et al. 2007) and stretch (Ditroilo et al. 2011). Stiffness (N/m) is a measure of the muscle's ability to resist an external force that modifies its shape and is calculated using the formula:

$$S = m_{probe}\,(\frac{a_{max}}{\Delta l})$$

The higher the N/m value, the stiffer the muscle (Gavronski et al. 2007).

Elasticity describes the tissue's ability to restore its shape after being deformed. Elasticity can be described by logarithmic decrement as the latter describes the dampening of the oscillation i.e. dissipation of mechanical energy in the tissue during an oscillation cycle. The smaller the decrement value, the smaller the subsequent dissipation of mechanical energy and the higher the elasticity (Gavronski et al. 2007).

Elasticity (logarithmic decrement) is expressed in arbitrary units as

$$[\theta = ln\left\{\frac{a_1}{a_3}\right\}]$$

2.5. Fat thickness measurements

Subcutaneous fat thickness was measured using ultrasound imaging to examine any relationship with Myoton parameters. A real-time ultrasound scanner (Aquila; Esaote Spa, Genova, Italy) with a 6-MHz linear transducer array (60-mm footprint) was used to take B-mode transverse images of subcutaneous tissue over the rectus femoris muscle at the same site as the Myoton measurements, with the participant resting in supine lying. The images were saved onto a flash card and downloaded to a computer for blinded analysis offline using MATLAB Version 10.1 (The MathWorks, Inc, Natick, MA). The Matlab algorithm (written by co-author MW) loaded ultrasound images automatically from a directory and prompted the operator to measure tissue thickness. Subcutaneous fat was measured as the distance from the skin (inferior border of layer of ultrasound gel) to the outside edge of superficial fascial layer over the muscle (Agyapong-Badu et al. 2014).

2.6 Reliability

Intra-session reliability between the first and second sets of 10 measurements was examined. A test-retest reliability study was also conducted in 58 participants (n=26 young, 32 older), on two different days, one week apart, at a similar time of day, in the hands of the present investigator (SAB).

2.7. Statistical Analysis

Data were imported from Microsoft Excel and analysed using SPSS 21 (SPSS Inc, Chicago, IL). The data were found to be normally distributed using the Shapiro-Wilk test. Descriptive statistics were used to summarise the data as means and standard deviations (SD). The mean values for each parameter (stiffness, elasticity and tone) were calculated from the responses to the 20 impulses delivered (two sets of 10). Within-session reliability was examined using intraclass correlation coefficient (ICC) model ICC 3,2. Reliability of measurements repeated on a second day was examined using ICC 3,1 to assess agreement, using the classification of (Fleiss 2007), in which ICC >0.75 is rated as excellent. Standard error of measurement (SEM) and minimal detectable change (MDC) were also used to assess precision of repeated measures. Independent sample and paired t-tests were used to assess gender, age-related and inter-muscle differences between the groups. Correlation coefficients for the relationship between the participants' age, subcutaneous fat thickness and each myometric parameter were also calculated using Pearson's correlation. Linear regression analysis was used to assess the influence of age, gender and fat thickness on Myoton parameters for the RF muscle only (gender classification; 0=females, 1=males). Significance levels were set at $p<0.05$.

3. Results

3.1. Reliability of Measurements Recorded Within-session and on Different Days

Within-session reliability was excellent for all parameters in both muscles (ICC 3,2 0.97–0.99). The SEM ranges were 0.03–5.49 for BB and 0.03–4.83 for RF (Appendix A). Reliability between days ranged from good to excellent and depended on age group and muscle property measured (ICC 3,1 BB; 0.72–0.93, RF; 0.68–0.94), as shown in Appendix B *(please see supplementary data on the journal website)*. The SEM ranges were 0.11–15.72 for BB and 0.11–12.57 for RF in both age groups. The MDC ranges for BB and RF were 0.29–43.58 and 0.31–34.82 respectively.

3.2. Muscle tone and mechanical properties

3.2.1. Effects of ageing

Older adults had significantly greater stiffness and lower elasticity (higher log decrement values) than younger adults for both BB and RF ($p<0.001$; Table 2; Table 3). Tone was also significantly greater in older adults for BB.

Table 2. Muscle tone and mechanical properties of biceps brachii and rectus femoris muscles

Myometric/ Ultrasound Parameter	Young adults (n=61) Males (n=34)	Females (n=27)	Older adults (n=62) Males (n=28)	Females (n=34)
BICEPS BRACHII				
Stiffness(N/m)				
Mean ± SD	213±24	215±28	288±56	302±50
Range	(171–273)	(172–294)	(194-399)	(219–425)
Elasticity(log D)				
Mean ± SD	1 ± 0.2	1.1 ± 0.2	1.5 ± 0.4	1.6 ± 0.3
Range	(0.7–1.4)	(0.8–1.7)	(1–2.5)	(1.2–2.3)
Tone (Hz)				
Mean ± SD	14 ± 0.8	13.6 ± 1.2	15.8 ± 1.7	15.4 ± 1.9
Range	(12.4–15.6)	(10.8–16.1)	(13–19.8)	(11.2–20.8)
RECTUS FEMORIS				
Stiffness(N/m)				
Mean ± SD	292±36	233± 35	328±29	311±42
Range	(228–410)	(177–323)	(254–398)	(236–417)
Elasticity(log D)				
Mean ± SD	1.3 ± 0.2	1.2 ± 0.2	1.6 ± 0.3	1.6 ± 0.2
Range	(0.9–1.7)	(0.7–1.5)	(1.2–2.3)	(1.1–2.0)
Tone(Hz)				
Mean ± SD	16.4 ± 1.6	13.6 ± 1.2	16.7 ± 1.6	14.9 ± 1.7
Range	(13.8–21.1)	(11.3–16.6)	(13.2–20.5)	(11.7–18.7)
Subcutaneous fat for RF (mm)				
Mean ± SD	4.1 ± 2.3	10.6 ±3.7	6.2 ±3.2	12.9 ±4
Range	(1.1-10.6)	(5.5-18.9)	(1.8-13.5)	(5.8-23.1)

SD=standard deviation; log D=log decrement; RF=rectus femoris

3.2.2. Effects of gender

For RF in the young adults, males had greater stiffness (mean 292.3 vs 232.8 N/m) and tone (16.4 vs 13.6 Hz), and lower elasticity (1.3 vs 1.2 log decrement) compared to females (Table 2). There were no gender differences for any of the three parameters for BB in both young and older groups (Table 3). In the older group, males again had greater stiffness (327.7 vs 310.5 N/m) and tone (16.7 vs 14.9 Hz) than females, while there was no difference in elasticity (both 1.6).The statistical differences for these gender effects are shown in Table 3.

Table 3. Significance test results for age and gender related differences in muscle tone and mechanical properties

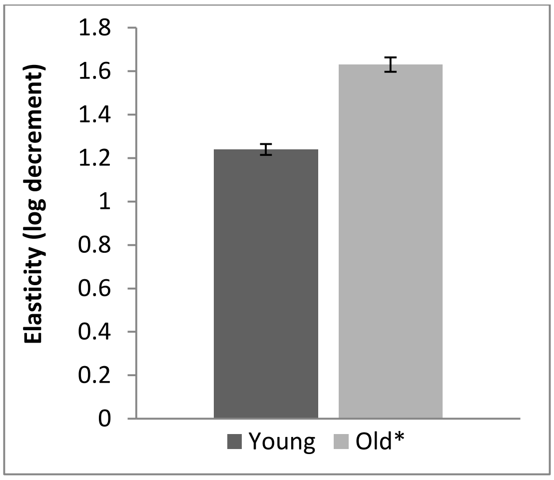
T-test analysis	Biceps Brachii			Rectus Femoris		
	E	T	S	E	T	S
All						
Young vs Old						
p value	0.00*	0.00*	0.00*	0.00*	0.28	0.00*
t	12.37	5.43	10.15	9.27	1.09	5.99
df	109	103	99	132	132	132
Mean difference	0.54	1.42	74.05	0.38	0.38	45.75
95% CI; Lower	0.44	0.90	59.57	0.29	−0.31	30.65
Upper	0.62	1.94	88.53	0.46	1.08	60.85
Males						
Young vs Old						
p value	0.00*	0.00*	0.00*	0.00*	0.48	0.00*
t	−7.35	−4.64	−6.36	−5.23	−0.71	−3.82
df	40	39	38	65	65	65
Mean difference	−0.52	−1.63	−71.91	−0.34	−0.30	−33.9
95% CI; Lower	−0.65	−2.33	−94.79	−0.46	−1.14	−51.62
Upper	−0.37	−0.91	−49.01	−0.20	0.54	−16.18
Females						
Young vs Old						
p value	0.00*	0.00*	0.00*	0.00*	0.01*	0.00*
t	−9.68	−3.49	−7.58	−8.73	−2.75	−6.79
df	65	62	61	65	65	65
Mean difference	−0.53	−1.35	−75.09	−0.44	−1.08	−68.79
95% CI; Lower	−0.64	−2.13	−94.89	−0.54	−1.87	−89.01
Upper	−0.42	−0.57	−55.29	−0.34	−0.29	−48.57
Young						
Male vs Females						
p value	0.06	0.12	0.69	0.02*	0.00*	0.00*
t	−1.85	1.55	−0.39	2.40	6.88	6.19
df	63	63	63	63	63	63
Mean difference	−0.08	0.38	−2.59	0.13	2.67	58.54
95% CI; Lower	−0.16	0.11	−15.74	0.021	1.89	39.64
Upper	0.01	0.86	10.56	0.22	3.44	77.44
Old						
Males vs Females						
p value	0.19	0.16	0.66	0.79	0.00*	0.01*
t	−1.30	1.40	−0.435	0.27	4.23	2.59
df	67	67	67	51	67	67
Mean difference	−0.09	0.65	−5.78	0.02	1.89	23.65
95% CI; Lower	−0.24	−0.27	−32.26	−0.11	0.99	5.42
Upper	0.052	1.57	20.70	0.14	2.77	41.88

E=elasticity; T=tone; S=stiffness; YG=young; df=degrees of freedom; CI=confidence interval; *significant at the 0.05 level (two-tailed)

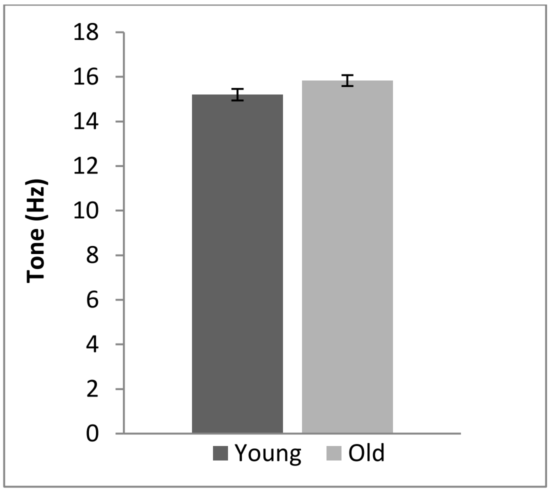
3.2.3 Differences in muscle parameters between age groups

When data were compared between age groups (males and females combined), significant differences were found for RF in elasticity and stiffness (Fig 3). In BB all three parameters differed significantly (Fig 4).

a.



b.



c.

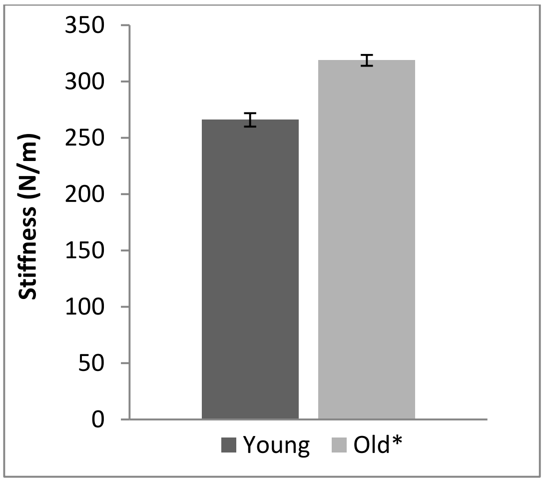
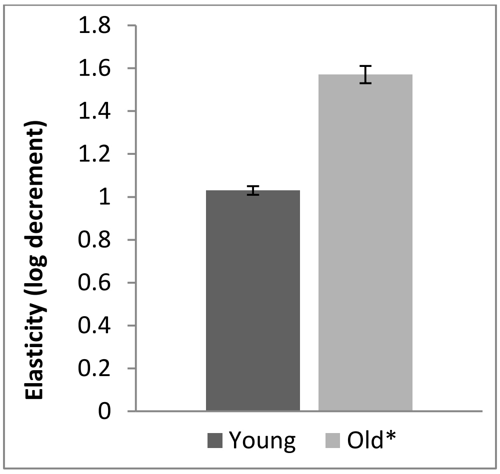
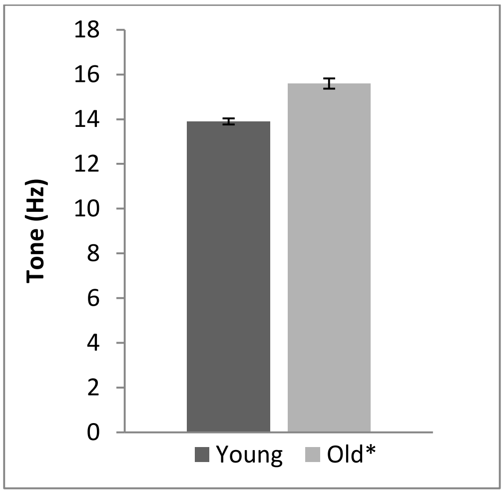


Figure 3. Differences in rectus femoris muscle parameters between healthy young and older adults measured using the MyotonPRO device. a) elasticity; b) tone; c) stiffness. *significant difference between groups p<0.001

a



b.



c.

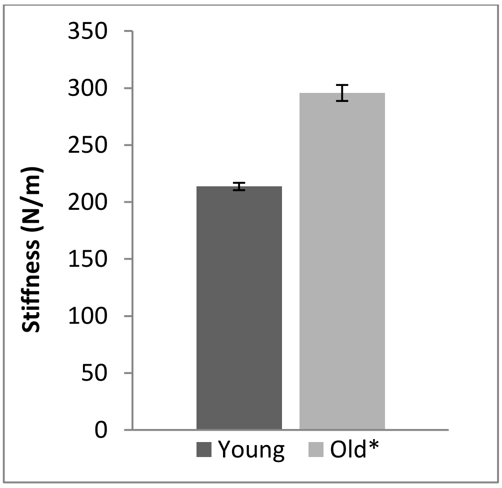


Figure 4. Differences in biceps brachii muscle parameters between healthy young and older adults measured using the MyotonPRO device. a) elasticity; b) tone; c) stiffness. *significant difference between groups $p<0.001$

3.2.4. Comparison between myometric parameters of the two muscles

Participants were categorised based on gender to assess inter-muscle differences using paired t-tests. Only stiffness was significantly higher for RF in females as shown in Table 4. For male participants, all three parameters recorded relatively higher means for RF compared to BB, and were significantly different when the two muscles were compared ($p<0.01$); Table 4).

Table 4. Significance test results for inter-muscle differences in tone and mechanical properties

	Biceps Brachii	Rectus Femoris	p-value	Mean difference	95% CI Lower-upper
Males (n=62)					
Stiffness (N/m)	247±56	308±37	0.00	-61.4	-74.5-(-48.4)*
Elasticity (log)	1.2±0.4	1.5±0.3	0.00	-0.2	-0.3-(-0.1)*
Tone (Hz)	14.9±1.5	16.5±1.6	0.00	-1.7	-2.2-(-1.2)*
Females (n=57)					
Stiffness (N/m)	261±60	274±55	0.02	-12.8	-23.1-(-2.6)*
Elasticity (log)	1.4±0.3	1.4±0.3	0.23	-0.04	-0.1-0.03
Tone (Hz)	14.6±1.8	14.3±1.7	0.28	0.23	-0.19-0.6

CI=confidence interval; *significant at the 0.05 level (two-tailed)

3.2.5. Percentage difference in Myoton parameters

The percentage differences between the young and older groups (Appendix B; *please see supplementary data on the journal website)* indicate the direction and magnitude of change with ageing. Ageing is associated with greater stiffness (+10–25%) and tone (+2–10%) and lower elasticity (–20–30%).

3.2.6 Relationships between muscle parameters and age, gender and subcutaneous fat (in RF)

Regression analysis for the RF muscle showed that age, gender and fat thickness were predictive ($p<0.001$) of the model for stiffness and tone parameters. Age was the only factor that was significantly predictive ($p<0.001$) of the model for elasticity, while fat thickness ($p=0.703$) and gender ($p=0.118$) were not predictive of elasticity. Overall the ANOVA results showed that all three models were good fit for the data ($p<0.001$). The details of the models from regression analysis are shown in Appendix D.

All three BB parameters showed significant correlations with age (log decrement, $r=0.76$, $p<0.001$, so elasticity negatively correlated with age; frequency (Hz), $r=0.52$, $p<0.001$, tone positively correlated; stiffness (N/m), $r=0.72$, $p<0.001$, stiffness positively correlated). For RF, only elasticity (log decrement, $r=0.65$, $p<0.001$) and stiffness (N/m, $r=0.53$, $p<0.001$) showed significant correlations, while there was no correlation for tone ($r=0.16$, $p=0.08$).

Correlation between Myoton parameters and fat thickness ranged from $r=-0.01$ to -0.49 within the four groups (Appendix E). Significant correlations were found for stiffness in older females only ($r=-0.38$; $p=0.04$), and for tone in young females ($r=-0.48$; $p=0.01$), older males ($r=-0.42$; $p=0.03$) and older females ($r=-0.49$; $p=0.01$). There were no significant correlations for elasticity.

4. Discussion

The present study was the first to examine the effect of ageing and gender on tone and mechanical properties of BB and RF muscles using Myoton technology. There were age related differences, and to a lesser extent gender differences, for both muscles and differences between the two muscles. The present findings provide comparative reference data from healthy males and females from young and older adult age groups. Specifically, the hypotheses for effects of age were supported for most

Myoton parameters in both muscles studied and both genders. The hypothesis for effect of gender on parameters was largely supported for the RF muscle and was rejected for the BB muscle.

4.1. Effects of ageing on Myoton Parameters

Muscle stiffness was significantly greater in older participants. The percentage difference in stiffness was similar for BB in both genders and RF in females (approximately 25%) but was less for RF in males (11%; Table Appendix B).

A previous study of RF (n=10, 5 females, 5 males, mean age 40 years, SD 13), using an earlier prototype, recorded mean stiffness of 268±31 N/m (Bizzini & Mannion 2003), which was lower than that for the present young males but higher than for young females (Table 2). Direct comparison with the present data is limited due to differences in age and they combined data for males and females. The present findings are consistent with laboratory based technology studies, which also found greater stiffness with ageing in the plantar flexors when measuring resistance to a single stretch (Blanpied & Smidt 1993) and in BB using sheer wave elastography (Eby et al. 2015).

Elasticity was significantly lower in the older groups in both muscles, with percentage differences of approximately 30% for BB in both genders but was less for RF (25% females, 19% males).

Muscle tone (Hz) was less variable between groups and increased with ageing but was only significant for BB (Tables 2&3). Percentage differences were relatively small compared with stiffness and elasticity (2-11%).

Correlation analysis showed that ageing was associated with stiffer, more toned and less elastic BB. For RF, ageing was also associated with stiffer and less elastic muscles but tone was not influenced by ageing in the present healthy participants. As well as ageing effects, the regression analysis for RF indicated effects of gender and subcutaneous fat (Appendix D) on stiffness and tone but not on elasticity, which was only influenced by age. These relationships are discussed below.

4.2. Gender Differences

The lack of differences between genders for BB in both age groups was unlike RF, where there were consistent gender differences in both age groups. Males had greater stiffness and tone, and lower elasticity than females (Table 2).

The greater stiffness in males than females for RF was consistent with findings in knee extensors using the free oscillation technique (Wang et al. 2015). The present lack of gender difference for BB may reflect differences between the muscles studied, as BB has previously been shown to have greater stiffness in females than males, using sheer wave elastography (Eby et al. 2015) and contrasting with knee extensor findings.

The present differences in RF Myoton parameters between genders could not be explained entirely by subcutaneous fat thickness. The approximate subcutaneous thicknesses were: young males 4mm, young females 11mm, older males 6mm, older females 13mm). From these values, genders might be expected to have greater differences in Myoton parameters than differences between age groups but this was not the case. Females within each age group had lower stiffness and tone than their male counterparts (Table 2) but this could not be attributed to greater subcutaneous fat because both older males and females had significantly greater stiffness and tone than the younger groups (Fig 3), despite having slightly greater fat thickness. The relationship between muscle parameters and fat thickness was therefore conflicting between the two scenarios. The systematic differences in stiffness and tone between genders is consistent with known differences in muscle characteristics e.g. in strength, where males are stronger than females (Narici & Maffulli 2010) with greater proportions of type 2 fibres and females have greater fatigue resistance, characteristic of type 1 fibres (Wüst et al. 2008).

The relative differences in RF tone and stiffness between gender and age may partially be accounted for by subcutaneous fat, but the same cannot be said about elasticity and parameters for BB which may not be directly related to subcutaneous fat thickness and these interrelationships require more detailed investigation.

The sex differences in muscle strength and fatigability outlined in the introduction may be reflective of fibre-type differences and neural activation (Wüst et al. 2008; Narici & Maffulli 2010). A recent review highlighted the lack of understanding of sex differences in neuromuscular function and fatigability, the underlying mechanisms and the functional consequences (Hunter 2014), so evidence-based conclusions on the mechanisms underlying Myoton parameters require further research alongside laboratory based technologies.

4.3 Relationship between Subcutaneous Fat and Muscle Parameters

Fröhlich-Zwahlen et al. (2014) suggested that subcutaneous fat influenced Myoton parameters. They used ultrasound imaging to measure fat thickness over RF and reported correlations for tone ($r=0.56$; $p<0.001$), stiffness ($r=-0.28$; $p<0.001$) and elasticity ($r=-0.21$) in 40 participants of different ages and genders. Although these correlation values were statistically significant, they were low to moderate. The present values were lower for tone and elasticity than those found by Fröhlich-Zwahlen et al. (2014), and were not significant for all parameters in the four groups (Appendix E). The previous study pooled the data from different age groups and genders, which did not allow for the effects of these different factors on the relationship with fat to be taken into account. Since subcutaneous fat thickness varies with age and gender (Table 2), a pooled correlation analysis would exaggerate the relationship between the muscle parameters and fat. This would be analogous with correlating another factor known to vary with age and gender that has no direct relationship with muscle, such as bone density, and finding that it significantly correlated with Myoton parameters. While fat thickness is associated with muscle parameters in certain cases, it is difficult to conclude that fat thickness influences muscle parameters directly.

4.4. Differences in Mechanical Parameters between Muscles

Parameters varied between the two muscles with gender. Values for stiffness and tone were greater, and elasticity lower, in RF than BB in males. In females, only stiffness was significantly greater in RF than BB ($p=0.02$), with tone and elasticity being similar between muscles (Table 4). The stiffer and less elastic RF in the healthy participants is in keeping with the greater decline in lower limb muscle characteristics with ageing than the upper limbs, e.g. strength (Traon et al. 2007; Samuel et al. 2012). For male participants, RF muscle was less elastic, stiffer and recorded higher tone compared to BB ($p<0.001$). The RF muscle which forms part of a major muscle in the body, the quadriceps, is a long bipennate muscle with two rows of muscle fibres to allow greater power but less range of motion, while BB is a small spindle shaped muscle with short tendinous structure. Nielsen et al. (2006) reported that the vastus lateralis muscle, another component of the quadriceps, contained more non-contractile components than supraspinatus muscle in the upper limb, using ultrasound imaging. It is possible that the overall greater tone and stiffness in the RF muscle observed in the present study could be as a result of the differences in non-contractile components in the lower limb muscle compared to those in the BB muscle. In the present study, intramuscular components were not assessed and this may be a useful measure to further understand the differences in mechanical properties for both muscles. The results for RF muscle in the current males corroborate reported greater quadriceps size and strength than in women (Young et al. 1985; Cooper et al. 2011a; Samuel et al. 2011).

4.5. Reliability of Measurements Within-session and Between-Days

The present reliability results are consistent with between day reliability studies of RF in healthy young (ICC 0.81-0.87); (Mullix et al. 2012) and older males (ICC 0.77-0.82); (Aird et al. 2012) using the MyotonPRO device. Reliability between days is important for monitoring changes over time. Based on excellent within-session reliability results, it can be concluded that the mean of one set of 10 measurements is as reliable as the mean of 2 sets of 10, so future studies could use one set of 10 measurements, particularly in field settings where time may be limited.

4.6. Limitations of the study

Relaxation was not measured objectively, e.g. using electromyography, so it was not possible to be certain that the muscles were in a resting state. The effect of ageing was only examined in two age bands (18-35 and 65-90 years), rather than across a range of age bands, to examine the progressive effects of age. Comparison of Myoton measurements with a laboratory based technology would have enabled the results to be interpreted more thoroughly in relation to muscle physiology.

4.7. Further research on ageing

The present findings warrant further research in different muscles to determine normative values across the age spectrum for comparison with clinical groups, and potentially establish an index in different healthy, clinical and sports cohorts. For example, how does lifestyle affect muscle tone and mechanical properties, and could such measures be used to assess health status and be predictive of mobility, independence and frailty?

Studies of neurological conditions using Myoton technology to measure abnormal muscle tone and the effects of interventions are emerging, e.g. stroke (Chuang et al. 2012) and PD (Rätsep & Asser 2011; Marusiak et al. 2012). Studies of Myoton parameters in musculoskeletal conditions, such as osteoarthritis and rheumatoid arthritis, may be worthwhile. The present findings on the effects of ageing need to be considered when studying neurological and musculoskeletal conditions that primarily affect older people.

5. Conclusions

5.1 Effects of ageing on muscle tone and mechanical properties has been documented in BB and RF using the novel hand-held MyotonPRO device. Stiffness was greater and elasticity lower in the older groups for both muscles. Tone was also greater for both genders in older adults for BB, and in females for RF, but there was no age difference in males for RF.

5.2 The effects of gender were less pronounced than ageing. There were no gender differences for any parameters for BB in either age group. For RF in the young adults, females had greater stiffness and elasticity and lower tone than males. In the older group, females had lower stiffness and tone than males.

5.3 All three parameters were greater in RF than BB in males. In females, only stiffness was greater in RF than BB, and tone and elasticity were similar.

5.4. Relationships between subcutaneous fat and muscle parameters were inconsistent between ageing and gender differences to allow a conclusion to be drawn about any direct effects of fat on muscle parameters.

5.5 Reference data for muscle tone, elasticity and stiffness have been provided for healthy young and older adults. Further data are needed in other age groups to produce a comprehensive data set with which to compare patients.

Conflict of interest statement

All authors declare no conflict of interest

Funding

This work was supported by the Ghana Education Trust Fund and Faculty of Health Sciences, University of Southampton, UK for funding a PhD studentship for SAB; and Arthritis Research UK [Grant ref: 20194] for MW, in the Arthritis Research UK Centre for Sport, Exercise and Osteoarthritis.

Acknowledgments

The authors thank participants and Aleko Peipsi, CEO of Myoton AS (Estonia) for loan of the MyotonPRO device and technical support.

Figure Legends

Figure 1. MyotonPRO technique for measurement of biceps brachii (BB) stiffness, tone, and elasticity

Figure 2. MyotonPRO technique for measurement of rectus femoris (RF) stiffness, tone and elasticity

Figure 3. Differences in rectus femoris muscle parameter between healthy young and older adults measured using the MyotonPRO device. a) elasticity; b) tone; c) stiffness. *significant difference $p<0.001$

Figure 4. Differences in biceps brachii muscle parameters between healthy young and older adults measured using the MyotonPRO device. a) elasticity; b) tone; c) stiffness. *significant difference between groups $p<0.001$

References

Aarrestad DD, Williams MD, Fehrer SC, Mikhailenok E and Leonard CT (2004) Intra- and interrater reliabilities of the Myotonometer when assessing the spastic condition of children with cerebral palsy. *Journal of Child Neurology* 19(11): 894-901

Agyapong-Badu S, Aird L, Bailey L, Mooney K, Mullix J, Warner M, Samuel D and Stokes M (2013) Interrater reliability of muscle tone, stiffness and elasticity measurements of rectus femoris and biceps brachii in healthy young and older males. *Working Papers in the Health Sciences* 1(4): 1-11

Agyapong-Badu S, Warner M, Samuel D, Narici M, Cooper C and Stokes M (2014) Anterior thigh composition measured using ultrasound imaging to quantify relative thickness of muscle and non-contractile tissue: a potential biomarker for musculoskeletal health. *Physiological Measurement* 35(10): 2165-76

Aird L, Samuel D and Stokes M (2012) Quadriceps muscle tone, elasticity and stiffness in older males: Reliability and symmetry using the MyotonPRO. *Archives of Gerontology and Geriatrics* 55(2): e31-e39

Bailey L, Samuel D, Warner M and Stokes M (2013) Parameters representing muscle tone, elasticity and stiffness of biceps brachii in healthy older males: symmetry and within-session reliability using the MyotonPRO. *J Neurol Disord* 1(1): 1-7

Baumgartner RN and Waters LW (2006) Sarcopenia and sarcopenic-obesity. IN*:* Pathy MS, Sinclair AJ and Morley JE (eds) *Principles and Practice of Geriatric Medicine.* Chichester: John Wiley & Sons Ltd 909-933

Bizzini M and Mannion AF (2003) Reliability of a new, hand-held device for assessing skeletal muscle stiffness. *Clin Biomech* 18(5): 459-61

Blanpied P and Smidt GL (1993) The Difference in Stiffness of the Active Plantarflexors Between Young and Elderly Human Females. *Journal of Gerontology* 48(2): M58-M63

Britton T (2004) Abnormalities of muscle tone and movement IN*:* Stokes M (ed) *Physical management in neurological rehabilitation.* 2nd Edition London: Elsevier Mosby 47-56

Chuang LL, Wu CY and Lin KC (2012) Reliability, validity and responsiveness of myotonometric measurement of muscle tone, elasticity and stiffness in patients with stroke. *Arch Phys Med Rehabil* 93(3): 532-540

Cooper R, Hardy R, Sayer AA, Ben-Shlomo Y, Birnie K, Cooper C, Craig L, Deary IJ and Al. E (2011a) Age and Gender Differences in Physical Capability Levels from Mid-Life Onwards: The Harmonisation and Meta-Analysis of Data from Eight UK Cohort Studies. *PLoS One* 6(11): e27899. doi:10.1371/journal.pone.0027899

Cooper R, Kuh D, Cooper C, Gale CR, Lawlor DA, Matthews F, Hardy R and Teams TFaHS (2011b) Objective measures of physical capability and subsequent health: a systematic review. *Age & Ageing* 40(1): 14–23

Cruz-Jentoft AJ, Baeyens JP, Bauer JM, Boirie Y, Cederholm T, Landi F, Martin FC, Michel JP, Rolland Y, Schneider SM, Topinková E, Vandewoude M and Zamboni M (2010) Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age and Ageing* 39(4): 412-423

Ditroilo M, Hunter A, Haslam S and De Vito G (2011) The effectiveness of two novel techniques in establishing the mechanical and contractile responses of the biceps femoris. *Physiol Measure* 32: 1315-1326

Dresner MA, Rose GH, Rossman PJ, Muthupillia R, Manduca A and Ehman RL (2001) Magnetic resonance elastography of skeletal muscle. *Journal of Magnetic Resonance Imaging* 13(2): 269-276

Eby SF, Cloud BA, Brandenburg JE, Giambini H, Song P, Chen S, Lebrasseur NK and An K-N (2015) Shear wave elastography of passive skeletal muscle stiffness: Influences of sex and age throughout adulthood. *Clinical Biomechanics* 30(1): 22-27

Fleiss J (2007) Reliability of measurement *The Design and Analysis of Clinical Experiments. .* New York: Wiley and Sons 1-33

Fleuren JFM, Voerman GE, Erren-Wolters CV, Snoek GJ, Rietman JS, Hermens HJ and Nene AV (2010) Stop using the Ashworth Scale for the assessment of spasticity. *Journal of Neurology, Neurosurgery & Psychiatry* 81(1): 46-52

Fröhlich-Zwahlen AK, Casartelli NC, Item-Glatthorn JF and Maffiuletti NA (2014) Validity of resting myotonometric assessment of lower extremity muscles in chronic stroke patients with limited hypertonia: A preliminary study. *Journal of Electromyography and Kinesiology* 24(5): 762-769

Gavronski G, Veraksits A, Vasar E and Maaroos J (2007) Evaluation of viscoelastic parameters of the skeletal muscles in junior triathletes. *Physiological Measurement* 28(6): 625-637

Goetz CG, Schrag WPBD, Langpeter MBSaE, Lewitt A, Olanow SFJJW, Martinez-Martin P, Lees A, Rascol O, Van Hilten B and Holloway GTSR (2003) The Unified Parkinson's Disease Rating Scale (UPDRS): Status and recommendations. *Movement Disorders* 18(7): 738-750

Hunter SK (2014) Sex differences in human fatigability: mechanisms and insight to physiological responses. *Acta Physiologica* 210(4): 768-789

Kararizou E, Manta P, Kalfakis N and Vassilopoulos D (2009) Age-related morphometric characteristics of human skeletal muscle in male subjects. *Pol J Pathol* 4: 186-188

Kubo K, Ikebukuro T, Yata H, Tsunoda N and Kanehisa H (2010) Effects of Training on Muscle and Tendon in Knee Extensors and Plantar Flexors in Vivo. *Journal of Applied Biomechanics* 26(3): 316-323

Manta P, Kalfakis N, Kararizou E, Vassilopoulos D and Papageorgiou K (1995) Distribution of muscle fibre types in human skeletal muscle fascicles: an autopsy study of three human muscles. *Funct Neurol* 10(3): 137-41

Marusiak J, Jaskólska A, Koszewicz M, Budrewicz S and Jaskólski A (2012) Myometry revealed medication-induced decrease in resting skeletal muscle stiffness in Parkinson's disease patients. *Clini Biomech* 27(6): 632-635

Mullix J, Warner M and Stokes M (2012) Testing muscle tone and mechanical properties of rectus femoris and biceps femoris using a novel hand held MyotonPRO device: relative ratios and reliability. *Working Papers in Health Sciences* 1(1): 1-8

Muraoka T, Chino K, Muramatsu T, Fukunaga T and Kanehisa H (2005) In vivo passive mechanical properties of the human gastrocnemius muscle belly. *J Biomech* 38: 1213-1219

Narici MV, Binzoni T, Hiltbrand E, Fasel J, Terrier F and Cerretelli P (1996) In vivo human gastrocnemius architecture with changing joint angle at rest and during graded isometric contraction. *J Physiol* 496: 287-297

Narici MV and Maffulli N (2010) Sarcopenia: Characteristic, mechanisms and functional significance. *British Medical Bulletin* 95(1): 139-159

Nielsen PK, Jensen BR, Darvann T, Jørgensen K and Bakke M (2006) Quantitative ultrasound tissue characterization in shoulder and thigh muscles – a new approach. *BMC Musculoskeletal Disorders* 7(2)

Rätsep T and Asser T (2011) Changes in viscoelastic properties of skeletal muscles induced by subthalamic stimulation in patients with Parkinson's disease. *Clin Biomech* 26(2): 213-217

Rosenberg IH (2011) Sarcopenia: Origins and Clinical Relevance. *Clinics in Geriatric Medicine* 27(3): 337-339

Samuel D, Rowe P, Hood V and Nicol A (2011) The biomechanical functional demand placed on knee and hip muscles of older adults during stair ascent and descent. *Gait & Posture* 34: 239-244

Samuel D, Wilson K, Martin HJ, Allen R, Sayer AA and Stokes M (2012) Age-associated changes in hand grip and quadriceps muscle strength ratios in healthy adults. *Aging Clinical and Experimental Research* 24(3): 245-50

Sayer AA, Syddall HE, Martin HJ, Dennison EM, Roberts HC and Cooper C (2006) Is grip strength associated with health-related quality of life?Findings from the Hertfordshire Cohort Study. *Age and Ageing* 35(4): 409-415

Scaglioni G, Narici MV, Maffiuletti NA, Pensini M and Martin A (2003) Effect of ageing on the electrical and mechanical properties of human soleus motor units activated by the H reflex and M wave. *J Physiol* 548: 649-661

Traon AP, Heer M, Narici MV, Rittweger J and Vernikos J (2007) From space to Earth: advances in human physiology from 20 years of bed rest studies (1986–2006). *Eur J Appl Physiol* 101: 143–194

Wang D, De Vito G, Ditroilo M, Fong DTP and Delahunt E (2015) A comparison of muscle stiffness and musculoarticular stiffness of the knee joint in young athletic males and females. *Journal of Electromyography and Kinesiology* 25(3): 495-500

Washburn RA, Smith KW, Jette AM and Janney CA (1993) The Physical Activity Scale for the Elderly (PASE): Development and evaluation. *Journal of Clinical Epidemiology* 46(2): 153-162

Wüst RCI, Morse CI, De Haan A, Jones DA and Degens H (2008) Sex differences in contractile properties and fatigue resistance of human skeletal muscle. *Experimental Physiology* 93(7): 843-850

Young A, Stokes M and Crowe M (1985) Size and strength of the quadriceps muscles in old and young men. *Clin Physiol* 5: 145-154

Appendices

Appendix A. Within-session reliability of measuring muscle tone and mechanical properties

		Participants (n=58)			
Myometric parameter		Mean±SD	ICC	95%CI	SEM
1	Biceps Brachii				
	Elasticity (LD)				
	M1	1.18 ± 0.2	0.97	0.947-0.98	0.03
	M2	1.17 ± 0.2			
	Tone (Hz)				
	M1	15.1 ± 2.1	0.99	0.989-0.996	0.21
	M2	15.1 ± 2.1			
	Stiffness (N/m)				
	M1	256.8±55.0	0.99	0.987-0.995	5.49
	M2	256.4±54.9			
2	Rectus Femoris				
	Elasticity (LD)				
	M1	1.2 ± 0.2	0.97	0.953-0.982	0.03
	M2	1.2 ± 0.2			
	Tone (Hz)				
	M1	15.0 ± 2.1	0.99	0.991-0.997	0.21
	M2	15.0 ± 2.1			
	Stiffness(N/m)				
	M1	261.7±48.5	0.99	0.989-0.996	4.83
	M2	263.5±48.2			

SD=standard deviation; ICC=Intraclass Correlation Coefficient; CI=confidence interval; SEM=Standard Error of Measurement; LD=Log Decrement; M1=first set of 10 measurements; M2=second set of 10 measurements

Appendix B. Between-day reliability of measuring muscle tone and mechanical properties

Myometric	Young adults (n=26)					Older adults (n=32)				
Parameters	Mean ± SD	ICC3,1	95%CI	SEM	MDC	Mean ± SD	ICC3,1	95%CI	SEM	MDC
Biceps Brachii										
Elasticity (LD)										
Day 1	1.1 ± 0.2	0.72	0.51- 0.85	0.11	0.29	1.6 ± 0.3	0.80	0.65 - 0.89	0.13	0.37
Day 2	1.2 ± 0.2					1.6 ± 0.3				
Tone (Hz)										
Day 1	14.9 ± 1.8	0.90	0.81- 0.95	0.57	1.58	15.4 ± 1.6	0.75	0.57 - 0.86	0.82	2.29
Day 2	15.0 ± 1.8					15.3 ± 1.7				
Stiffness (N/m)										
Day 1	250 ± 51	0.93	0.87- 0.97	13.3	36.9	287 ± 51	0.89	0.79 - 0.94	15.7	43.6
Day 2	251 ± 50					288 ± 44				
Rectus Femoris										
Elasticity (LD)										
Day 1	1.3 ± 0.2	0.68	0.4 - 0.83	0.11	0.31	1.6 ± 0.2	0.76	0.58 - 0.87	0.12	0.34
Day 2	1.3 ± 0.2					1.7 ± 0.3				
Frequency (Hz)										
Day 1	15.2 ± 1.9	0.94	0.89- 0.97	0.45	1.25	15.5 ± 1.9	0.92	0.86 - 0.96	0.54	1.49
Day 2	15.1 ± 1.8					15.4 ± 1.9				
Stiffness (N/m)										
Day 1	270 ± 47	0.93	0.86- 0.96	12.3	34.0	311 ± 41	0.90	0.82 - 0.95	12.6	34.8
Day 2	270 ± 46					309 ± 38				

SD=Standard Deviation; ICC=Intraclass Correlation Coefficient; CI=Confidence Interval; SEM=Standard Error of Measurement; MDC=Minimal Detectable Change; LD=Log Decrement

Appendix C. Percentage differences in Myometric parameters with Ageing

	Percentage difference from young to older group			
	Biceps Brachii		Rectus Femoris	
	Males	Females	Males	Females
Stiffness	+25	+26	+11	+23
Elasticity	−33	−31	−19	−25
Tone	+11	+9	+2	+8

NB. The negative difference for elasticity was due to the greater values for log decrement in the older groups

Appendix D. Regression analysis to examine the influence of age, gender and subcutaneous fat thickness on Myoton parameters for the rectus femoris muscle combining all four groups of participants

Muscle parameter	Regression equation x - fat thickness +/-age + gender	R^2	Adjusted R^2	F value	P value
Stiffness	246.62-32.36* + 1.25 +18.92*	0.51	0.49	F (3,113) = 38.4	<0.001
Tone	15.31-1.884* +0.025* +1.013*	0.46	0.45	F (3,113) = 32.1	<0.001
Elasticity	1.012 – 0.024 +0.008* +0.092	0.45	0.43	F (3,113) = 30.4	<0.001

* =significant at 0.05 level (2 tailed)

Appendix E. Correlation between Myometric parameters and fat thickness in young and old males and females

	Young males (n=33)	Young females (n=26)	Older males (n=28)	Older females (n=30)
Stiffness (N/m)				
r	-0.11	-0.39	-0.36	-0.38
p-value	0.544	0.052	0.061	**0.041***
Tone (Hz)				
r	-0.25	-0.48	-0.42	-0.49
p-value	0.159	**0.013***	**0.025***	**0.006***
Elasticity (log D)				
r	-0.12	-0.02	-0.01	-0.08
p-value	0.513	0.929	0.943	0.659

r=Pearson correlation coefficient; log D=log decrement; *significant correlation at 0.05 level (2 tailed)